

=&gt; d ibib abs ind l3 1-2

L3 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:620665 HCAPLUS

DOCUMENT NUMBER: 135:300288

TITLE: A sensitive immunochemical assay for measuring the concentration of the activated protein C-protein C inhibitor complex in plasma: use of a catcher antibody specific for the complexed/cleaved form of the inhibitor

AUTHOR(S): Strandberg, Karin; Kjellberg, Margareta; Knebel, Richard; Lilja, Hans; **Stenflo, Johan**

CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Malmo, Lund University, Malmo, S-20502, Swed.

SOURCE: Thrombosis and Haemostasis (2001), 86(2), 604-610  
CODEN: THHADQ; ISSN: 0340-6245

PUBLISHER: Schattauer GmbH

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Activated protein C (APC) is a **serine proteinase** that regulates blood coagulation. In plasma it is inhibited mainly by the protein C inhibitor (PCI). The plasma concns. of APC-PCI complex is increased in hyper-coagulative states such as deep venous thrombosis. Formation of the APC-PCI complex induces a drastic conformational change in PCI that exposes new epitopes (neoepitopes) on the mol. We have devised a simple immunofluorometric sandwich assay for measurements of the concns. of APC-PCI complex, employing as the catcher, a **monoclonal antibody** that has a high affinity ( $K_D = 4 + 10^{-11}M$ ) for a complexation-specific neoepitope that is expressed on PCI. A **monoclonal antibody** against protein C is employed as the tracer. The method gives a linear dose-response curve (0.06-50  $\mu g/l$ ), has a low detection limit (0.06  $\mu g/l$ ) and no crossreactivity with native PCI at physiol. plasma concns. We have now determined the concentration of the APC-PCI complex in healthy individuals.

CC 7-1 (Enzymes)

Section cross-reference(s): 13, 14

ST activated protein C inhibitor complex immunoassay blood

IT Blood coagulation

Blood plasma

Immunoassay

(immunochem. assay for measuring concentration of activated protein

C-protein

C inhibitor complex in plasma)

IT Antibodies

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)

(monoclonal, M36; immunochem. assay for measuring concentration of activated protein C-protein C inhibitor complex in plasma)

IT Dissociation constant

(of antigen-antibody complex; immunochem. assay for measuring concentration

of

activated protein C-protein C inhibitor complex in plasma)

IT Thrombosis

(venous; immunochem. assay for measuring concentration of activated protein C-protein C inhibitor complex in plasma)

IT 42617-41-4D, Blood-coagulation factor XIVa, complex with protein C inhibitor 139466-48-1D, Protein C inhibitor, complex with activated protein C

RL: ANT (Analyte); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC

(Process)

(immunochem. assay for measuring concentration of activated protein C-protein

C inhibitor complex in plasma)

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:836206 HCAPLUS

DOCUMENT NUMBER: 134:143678

TITLE: Activated Protein C-Protein C Inhibitor Complex Formation: Characterization of a Neopeptide Provides Evidence for Extensive Insertion of the Reactive Center Loop

AUTHOR(S): Strandberg, Karin; Kjellberg, Margareta; Erb, Eva-Maria; Persson, Ulla; Mosher, Deane F.; Villoutreix, Bruno O.; **Stenflo, Johan**

CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Malmo, S-205, Swed.

SOURCE: Biochemistry (2000), 39(51), 15713-15720

CODEN: BICHAW; ISSN: 0006-2960

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Protein C inhibitor, a **serine proteinase** inhibitor (serpin), is the physiol. most important inhibitor of activated protein C. We have made a **monoclonal antibody** (M36) that binds with equally high affinity to an epitope present in activated protein C-protein C inhibitor complexes and cleaved loop-inserted protein C inhibitor. Insertion of a synthetic N-acetylated tetradecapeptide (corresponding to residues P1-P14 of the reactive center loop) into  $\beta$ -sheet A of the uncleaved inhibitor also exposed the epitope. The antibody had no apparent affinity for native uncleaved inhibitor or for the free peptide. Synthetic P1-P14 analogs, with Arg P13 or Ala P9 substituted to the residues found in mouse protein C inhibitor (Thr and Ile, resp.), were also inserted in  $\beta$ -sheet A. The Arg P13/Thr substitution led to a greatly impaired reactivity with the antibody, whereas the Ala P9/Ile mutation resulted in a modest loss of proline-glutamate reactivity with the antibody. These results indicate that complex formation leads to insertion of the reactive center loop in  $\beta$ -sheet A from Arg P14 and presumably beyond Ala P9. Moreover, to the best of our knowledge, this is the first instance where the neopeptide of a complexation-specific **monoclonal antibody** has been localized to the loop-inserted part of  $\beta$ -sheet A, the part of the serpin where the complexation-induced conformational change is most conspicuous.

CC 7-2 (Enzymes)

ST protein C inhibitor complex conformation model

IT Molecular modeling

(activated protein C-protein C inhibitor complex formation)

IT Conformation

(protein; activated protein C-protein C inhibitor complex formation)

IT 139466-48-1, Protein C inhibitor

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)  
(complexes with APC; activated protein C-protein C inhibitor complex formation)

IT 42617-41-4, Blood-coagulation factor XIIVa

RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)

(complexes with PCI; activated protein C-protein C inhibitor complex formation)

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=&gt; d que stat 18

L4 1 SEA FILE=REGISTRY ABB=ON "PROTEIN C INHIBITOR"/CN  
 L5 1 SEA FILE=REGISTRY ABB=ON "SERINE PROTEINASE"/CN  
 L6 101819 SEA FILE=HCAPLUS ABB=ON ?MONOCLON?(W)?ANTIBOD?  
 L7 207 SEA FILE=HCAPLUS ABB=ON L6 AND (L5 OR ?SERINE?(W)?PROTEIN?)  
 L8 6 SEA FILE=HCAPLUS ABB=ON L7 AND (L4 OR ?PROTEIN?(W)C(W)?INHIBIT  
 ? OR ?ALPHA1?(W)?ANTITRYPSIN?)

=&gt; d ibib abs 18 1-6

L8 ANSWER 1 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:86508 HCAPLUS

DOCUMENT NUMBER: 136:323406

TITLE: Evaluation of oxidized alpha-1-antitrypsin in blood as  
 an oxidative stress marker using anti-oxidative  
 alpha-1-AT **monoclonal antibody**

AUTHOR(S): Ueda, Masashi; Mashiba, Shinichi; Uchida, Kazuo

CORPORATE SOURCE: Kyoto Medical Science Laboratory Incorporation,  
 Fushimi-ku, Hazukashi, Kyoto, 612-8486, Japan

SOURCE: Clinica Chimica Acta (2002), 317(1-2), 125-131

CODEN: CCATAR; ISSN: 0009-8981

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Background: alpha-1-AT is a 52-kDa acute-phase protein and a typical  
**serine proteinase** inhibitor, which is present in human  
 serum. In vivo, the inhibitor prevents tissue damage by inactivating  
 proteinases, such as elastase, that are released from activated  
 neutrophils in the presence of inflammation. Methods: the authors  
 obtained a **monoclonal antibody** against oxidized  
 alpha-1-AT(3F4) using chloramine T-oxidized alpha-1-AT as the antigen.  
 Results: This antibody did not react with either the native alpha-1-AT or  
 the elastase-alpha-1-AT complex. However, it reacted with alpha-1-AT  
 oxidized by various oxidants and peroxide lipid. The oxidized alpha-1-AT  
 is a polymer with a mol. mass of 100-200 kDa in addition to the 52-kDa  
 protein that corresponds to the native alpha-1-AT in sera. In vitro  
 evaluations reveal that fatty acids are involved in the polymerization  
 Furthermore, the concns. of oxidized alpha-1-AT in the sera of patients  
 with inflammatory and rheumatoid diseases were higher than those in  
 healthy subjects. Conclusions: the authors considered that 3F4 is an  
 effective antibody that can specifically recognize oxidized alpha-1-AT, a  
 marker of oxidative stress.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS  
 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:620665 HCAPLUS

DOCUMENT NUMBER: 135:300288

TITLE: A sensitive immunochemical assay for measuring the  
 concentration of the activated protein C-  
**protein C inhibitor**

complex in plasma: use of a catcher antibody specific  
 for the complexed/cleaved form of the inhibitor

AUTHOR(S): Strandberg, Karin; Kjellberg, Margareta; Knebel,  
 Richard; Lilja, Hans; Stenflo, JohanCORPORATE SOURCE: Department of Clinical Chemistry, University Hospital,  
 Malmo, Lund University, Malmo, S-20502, Swed.

SOURCE: Thrombosis and Haemostasis (2001), 86(2), 604-610

CODEN: THHADQ; ISSN: 0340-6245

PUBLISHER: Schattauer GmbH

2/9/99

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Activated protein C (APC) is a **serine proteinase** that regulates blood coagulation. In plasma it is inhibited mainly by the **protein C inhibitor** (PCI). The plasma concns. of APC-PCI complex is increased in hyper-coagulative states such as deep venous thrombosis. Formation of the APC-PCI complex induces a drastic conformational change in PCI that exposes new epitopes (neoepitopes) on the mol. We have devised a simple immunofluorometric sandwich assay for measurements of the concns. of APC-PCI complex, employing as the catcher, a **monoclonal antibody** that has a high affinity ( $KD = 4 + 10^{-11}M$ ) for a complexation-specific neoepitope that is expressed on PCI. A **monoclonal antibody** against protein C is employed as the tracer. The method gives a linear dose-response curve ( $0.06-50 \mu g/l$ ), has a low detection limit ( $0.06 \mu g/l$ ) and no crossreactivity with native PCI at physiol. plasma concns. We have now determined the concentration of the APC-PCI complex in healthy individuals.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:836206 HCAPLUS

DOCUMENT NUMBER: 134:143678

TITLE: Activated Protein C-**Protein C Inhibitor** Complex Formation: Characterization of a Neoepitope Provides Evidence for Extensive Insertion of the Reactive Center Loop

AUTHOR(S): Strandberg, Karin; Kjellberg, Margareta; Erb, Eva-Maria; Persson, Ulla; Mosher, Deane F.; Villoutreix, Bruno O.; Stenflo, Johan

CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Malmö, S-205 Swed.

SOURCE: Biochemistry (2000), 39(51), 15713-15720

CODEN: BICHAW; ISSN: 0006-2960

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Protein C inhibitor**, a **serine proteinase** inhibitor (serpin), is the physiol. most important inhibitor of activated protein C. We have made a **monoclonal antibody** (M36) that binds with equally high affinity to an epitope present in activated protein C-**protein C inhibitor** complexes and cleaved loop-inserted **protein C inhibitor**. Insertion of a synthetic N-acetylated tetradecapeptide (corresponding to residues P1-P14 of the reactive center loop) into  $\beta$ -sheet A of the uncleaved inhibitor also exposed the epitope. The antibody had no apparent affinity for native uncleaved inhibitor or for the free peptide. Synthetic P1-P14 analogs, with Arg P13 or Ala P9 substituted to the residues found in mouse **protein C inhibitor** (Thr and Ile, resp.), were also inserted in  $\beta$ -sheet A. The Arg P13/Thr substitution led to a greatly impaired reactivity with the antibody, whereas the Ala P9/Ile mutation resulted in a modest loss of proline-glutamate reactivity with the antibody. These results indicate that complex formation leads to insertion of the reactive center loop in  $\beta$ -sheet A from Arg P14 and presumably beyond Ala P9. Moreover, to the best of our knowledge, this is the first instance where the neoepitope of a complexation-specific **monoclonal antibody** has been localized to the loop-inserted part of  $\beta$ -sheet A, the part of the serpin where the complexation-induced conformational change is most conspicuous.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 4 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:573838 HCAPLUS  
DOCUMENT NUMBER: 133:176177  
TITLE: **Monoclonal antibody to protein C inhibitor**

INVENTOR(S): Stenflo, Johan  
PATENT ASSIGNEE(S): Protease Ab, Swed.  
SOURCE: PCT Int. Appl., 31 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent  
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE     |
|---|------|----------|-----------------|----------|
| WO 2000047626   | A1   | 20000817 | WO 2000-SE210   | 20000203 |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |      |          |                 |          |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG  |      |          |                 |          |
| EP 1151013  | A1   | 20011107 | EP 2000-906836  | 20000203 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO   |      |          |                 |          |

PRIORITY APPLN. INFO.: SE 1999-431 A 19990209  
WO 2000-SE210 W 20000203

AB The author discloses the preparation and characterization of a **monoclonal antibody** that exhibits reactivity with either inactivated **protein C inhibitor** (PCI) or inhibitor in complex with activated protein C (APC). Using a fluorescent immunoassay (DELPHIA) the **monoclonal antibody** was shown to be suitable for monitoring the concentration of APC:PCI complexes in human plasma.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1994:211319 HCAPLUS  
DOCUMENT NUMBER: 120:211319  
TITLE: **Complex formation between protein C inhibitor and prostate-specific antigen in vitro and in human semen**

AUTHOR(S): Christensson, Anders; Lilja, Hans  
CORPORATE SOURCE: Dep. Clin. Chem., Malmoe Gen. Hosp., Malmoe, Swed.  
SOURCE: European Journal of Biochemistry (1994), 220(1), 45-53  
CODEN: EJBCAI; ISSN: 0014-2956

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB **Protein C inhibitor** (PCI), a **serine -proteinase** inhibitor first purified from human blood plasma, occurs at high concns. (3-4  $\mu$ M) in seminal fluid in both a high-mol.-mass and low-mol.-mass form. Immunochem. data have previously

suggested that PCI in seminal plasma forms complexes with the most abundant **serine proteinase** in semen, prostate-specific antigen (PSA). To provide a structural characterization of the PCI target, immunodetected as PSA, a procedure was developed to isolate low-mol.-mass and high-mol.-mass-forms of PCI from seminal fluid. The high-mol.-mass form of PCI, recognized by **monoclonal antibodies** against PSA, was dissociated by alkaline treatment into the low-mol.-mass form of PCI and a 33-kDa protein identified as PSA by 25 conclusive steps of N-terminal sequence anal. The authors developed a sensitive immunofluorometric assay (IFMA) to measure PCI-PSA complexes in body fluids and investigated the rate at which purified PSA may form complexes with purified PCI. Formation of complexes detected by this IFMA and the appearance of SDS-stable approx. 90-kDa complexes paralleled loss of PSA activity recorded with chromogenic substrates. The rate of complex formation was slow compared to that reported for PCI and activated protein C, but was enhanced up to sixfold in the presence of heparin. Less than 10% of the initial PSA activity remained after 3 h incubation with a sevenfold molar excess of PCI and in the presence of heparin. In freshly collected ejaculates, the rate of PCI-PSA complex formation measured by IFMA was similar to that observed between the purified proteins, and paralleled the appearance of SDS-stable complexes by immunoblotting. During gel dissoln. in freshly collected ejaculates, approx. 40% of immunodetected PCI becomes complexed to PSA. Although PCI is a slow inhibitor of PSA, complexes between PCI and PSA are detected at levels that correspond to an inactivation of up to 5% of the PSA activity in the ejaculate.

L8 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN  
 ACCESSION NUMBER: 1993:531522 HCAPLUS  
 DOCUMENT NUMBER: 119:131522  
 TITLE: Serine protease derived-polypeptides, anti-peptide antibodies, and systems and therapeutic methods for inhibiting coagulation  
 INVENTOR(S): Griffin, John H.; Mesters, Rolf M.  
 PATENT ASSIGNEE(S): Scripps Research Institute, USA  
 SOURCE: PCT Int. Appl., 149 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

| PATENT NO.   | KIND | DATE     | APPLICATION NO. | DATE     |
|--|------|----------|-----------------|----------|
| WO 9309804   | A1   | 19930527 | WO 1992-US10242 | 19921118 |
| W: CA, JP  |      |          |                 |          |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE |      |          |                 |          |
| US 5679639   | A    | 19971021 | US 1994-295411  | 19940822 |
| US 5968751   | A    | 19991019 | US 1997-955471  | 19971021 |
| PRIORITY APPLN. INFO.:   |      |          | US 1991-793989  | 19911118 |
|  |      |          | US 1994-295411  | 19940822 |

AB Peptides and anti-peptide antibodies are disclosed which can inhibit serine protease activity. In particular, peptides and anti-peptide antibodies derived from the blood coagulation serine proteases Factor VIIa, Factor IXa, Factor Xa, Factor XIa, thrombin, and plasma kallikrein are described that are capable of inhibiting coagulation. The peptides and antibodies are useful in methods and systems for inhibiting serine proteases, end especially for inhibiting blood coagulation processes mediated by serine proteases in vitro or in a human patient. Production of polyclonal and

**monoclonal antibodies** to protein C fragments is described; activity of the peptides and antibodies of the invention (peptide sequences included) is demonstrated in a variety of coagulation-related assays.



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L4 1 SEA FILE=REGISTRY ABB=ON "PROTEIN C INHIBITOR"/CN  
 L5 1 SEA FILE=REGISTRY ABB=ON "SERINE PROTEINASE"/CN  
 L6 101819 SEA FILE=HCAPLUS ABB=ON ?MONOCLON?(W)?ANTIBOD?  
 L7 207 SEA FILE=HCAPLUS ABB=ON L6 AND (L5 OR ?SERINE?(W)?PROTEIN?)  
 L8 6 SEA FILE=HCAPLUS ABB=ON L7 AND (L4 OR ?PROTEIN?(W)C(W)?INHIBIT  
 ? OR ?ALPHA1?(W)?ANTITRYPSIN?)  
 L9 15 SEA L8  
 L10 7 DUP REMOV L9 (8 DUPLICATES REMOVED)

=&gt; d ibib abs 110 1-7

L10 ANSWER 1 OF 7 MEDLINE on STN  
 ACCESSION NUMBER: 2001351700 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 11415941  
 TITLE: Anti-proteinase 3 antibody activation of neutrophils can be inhibited by ~~alpha1-antitrypsin~~  
 AUTHOR: Rooney C P; ~~Raggatt C~~; Coakley R; McElvaney N G; O'Neill S J  
 CORPORATE SOURCE: Division of Respiratory Research, Department of Medicine, Royal College of Surgeons in Ireland Education and Research Centre, Beaumont Hospital, Dublin, Republic of Ireland.  
 SOURCE: American journal of respiratory cell and molecular biology, (2001 Jun) 24 (6) 747-54.  
 Journal code: 8917225. ISSN: 1044-1549.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200107  
 ENTRY DATE: Entered STN: 20010730  
 Last Updated on STN: 20010730  
 Entered Medline: 20010726

AB Wegener's granulomatosis (WG) is classically associated with the presence of cytoplasmic antineutrophil cytoplasmic autoantibodies (c-ANCA). Proteinase 3 (PR3), the target antigen for c-ANCA, is inhibited by the antiprotease ~~alpha1-antitrypsin~~ (A1AT), and recent studies have demonstrated that WG patients who are A1AT-deficient have a worse clinical course, suggesting that a protease-antiprotease imbalance may play a role in WG. We evaluated the effect of A1AT on anti-PR3 antibody-induced activation of neutrophils. The neutrophil was chosen because of its central role in the pathogenesis of WG. Isolated neutrophils from healthy controls were incubated with tumor necrosis factor (TNF)-alpha to induce surface expression of PR3. Subsequently, they were stimulated with a **monoclonal antibody** to PR3, resulting in a significant increase in respiratory burst. Addition of A1AT (1 mg/ml) to the TNF-alpha-primed cells before the addition of the anti-PR3 antibody resulted in a 47% reduction in anti-PR3 antibody-induced activation. A1AT mediated this inhibitory action by preventing anti-PR3 antibody binding to PR3 on the cell, thereby preventing the PR3-Fc gamma R11a cross-linkage required for cell activation. Further, anti-PR3 antibody-induced activation of neutrophils from WG patients can be reduced by 56% with A1AT. These data suggest that protease-antiprotease interactions may play a pivotal role in neutrophil activation in WG.

L10 ANSWER 2 OF 7 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 2001478497 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 11522010  
 TITLE: A sensitive immunochemical assay for measuring the

concentration of the activated protein C-**protein C inhibitor** complex in plasma: use of a catcher antibody specific for the complexed/cleaved form of the inhibitor.

AUTHOR: Strandberg K; Kjellberg M; Knebel R; Lilja H; Stenflo J  
 CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Malmö, Lund University, Sweden.  
 SOURCE: Thrombosis and haemostasis, (2001 Aug) 86 (2) 604-10. Journal code: 7608063. ISSN: 0340-6245.  
 PUB. COUNTRY: Germany: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200208  
 ENTRY DATE: Entered STN: 20010828  
 Last Updated on STN: 20020803  
 Entered Medline: 20020802

AB Activated protein C (APC) is a **serine proteinase** that regulates blood coagulation. In plasma it is inhibited mainly by the **protein C inhibitor** (PCI). The plasma concentrations of APC-PCI complex is increased in hypercoagulative states such as deep venous thrombosis. Formation of the APC-PCI complex induces a drastic conformational change in PCI that exposes new epitopes (neoepitopes) on the molecule. We have devised a simple immunofluorometric sandwich assay for measurements of the concentrations of APC-PCI complex, employing as the catcher, a **monoclonal antibody** that has a high affinity ( $K(D) = 4 \times 10^{-11}$  M) for a complexation-specific neoepitope that is expressed on PCI. A **monoclonal antibody** against protein C is employed as the tracer. The method gives a linear dose-response curve (0.06-50 microg/l), has a low detection limit (0.06 microg/l) and no crossreactivity with native PCI at physiologic plasma concentrations. We have now determined the concentration of the APC-PCI complex in healthy individuals.

L110 ANSWER 3 OF 7 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: 2000-646916 [62] WPIDS  
 DOC. NO. NON-CPI: N2000-479440  
 DOC. NO. CPI: C2000-195620  
 TITLE: Novel **monoclonal antibody** with specific affinity for **serine proteinase -serine proteinase** inhibitor complexes, and for cleaved uncomplexed inhibitors, useful for monitoring systems involving **protein C inhibitor**.  
 DERWENT CLASS: B04 D16 K08 S03  
 INVENTOR(S): STENFLO, J  
 PATENT ASSIGNEE(S): (PROT-N) PROTEASE AB  
 COUNTRY COUNT: 91  
 PATENT INFORMATION:

| PATENT NO   | KIND | DATE     | WEEK      | LA | PG |
|---|------|----------|-----------|----|----|
| WO 2000047626   | A1   | 20000817 | (200062)* | EN | 30 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL<br>OA PT SD SE SL SZ TZ UG ZW<br>W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES<br>FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS<br>LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL<br>TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW |      |          |           |    |    |
| AU 2000028393   | A    | 20000829 | (200062)  |    |    |

EP 1151013 A1 20011107 (200168) EN  
 R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT  
 RO SE SI

## APPLICATION DETAILS:

| PATENT NO     | KIND | APPLICATION    | DATE     |
|---------------|------|----------------|----------|
| WO 2000047626 | A1   | WO 2000-SE210  | 20000203 |
| AU 2000028393 | A    | AU 2000-28393  | 20000203 |
| EP 1151013    | A1   | EP 2000-906836 | 20000203 |
|               |      | WO 2000-SE210  | 20000203 |

## FILING DETAILS:

| PATENT NO     | KIND        | PATENT NO     |
|---------------|-------------|---------------|
| AU 2000028393 | A Based on  | WO 2000047626 |
| EP 1151013    | A1 Based on | WO 2000047626 |

PRIORITY APPLN. INFO: SE 1999-431 19990209

AN 2000-646916 [62] WPIDS

AB WO 200047626 A UPAB: 20001130

NOVELTY - **Monoclonal antibody** (I) with specific affinity for both a complex between a **serine proteinase** (SP) and a SP inhibitor, and a cleaved uncomplexed form of the inhibitor but no specific affinity for the inhibitor in its uncleaved and uncomplexed form, is new

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) preparation of (I) comprising immunizing an animal with a mixture of a SP and a SP inhibitor complex and a cleaved form of the inhibitor, and screening for and isolating (I);

(2) an immunoassay for monitoring the activity of systems involving **protein C inhibitor**, comprising (I); and

(3) a kit for qualitative or quantitative determination of the activity of systems involving **protein C inhibitor** comprising (I).

USE - The antibodies are useful for monitoring systems involving **protein C inhibitor** (especially as part of an immunoassay), especially the diagnosis of venous thrombosis, arterial thrombosis, embolism, coronary infraction, disseminated intravascular coagulation or disorders involving lupus anticoagulants (claimed).

DESCRIPTION OF DRAWING(S) - The diagram shows affinity chromatograms for activated protein C (APC)-complexed (A), cleaved (B), and native (C) **protein C inhibitor** (PCI) respectively, obtained on a gel column onto which the **monoclonal antibody** M36 was immobilized. The continuous line represents absorbance and the 'o-o' line represents fluorescence. The early eluting peak in (A) consists of UV absorbing low molecular weight compounds and APC from cleaved complexes.

Dwg.1/3

L10 ANSWER 4 OF 7

ACCESSION NUMBER: 2001116714 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11123896

TITLE: Activated protein C-protein C

**inhibitor** complex formation: characterization of a neopeptide provides evidence for extensive insertion of the reactive center loop.

DUPLICATE 2

AUTHOR: Strandberg K; Kjellberg M; Erb E M; Persson U; Mosher D F;  
Villoutreix B O; Stenflo J  
CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital,  
Malmo, Lund University, S-205 Malmo, Sweden.  
SOURCE: Biochemistry, (2000 Dec 26) 39 (51) 15713-20.  
Journal code: 0370623. ISSN: 0006-2960.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200102  
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AB **Protein C inhibitor, a serine proteinase inhibitor** (serpin), is the physiologically most important inhibitor of activated protein C. We have made a **monoclonal antibody** (M36) that binds with equally high affinity to an epitope present in activated protein C-**protein C inhibitor** complexes and cleaved loop-inserted **protein C inhibitor**. Insertion of a synthetic N-acetylated tetradecapeptide (corresponding to residues P1-P14 of the reactive center loop) into beta-sheet A of the uncleaved inhibitor also exposed the epitope. The antibody had no apparent affinity for native uncleaved inhibitor or for the free peptide. Synthetic P1-P14 analogues, with Arg P13 or Ala P9 substituted to the residues found in mouse **protein C inhibitor** (Thr and Ile, respectively), were also inserted in beta-sheet A. The Arg P13/Thr substitution led to a greatly impaired reactivity with the antibody, whereas the Ala P9/Ile mutation resulted in a modest loss of reactivity with the antibody. These results indicate that complex formation leads to insertion of the reactive center loop in beta-sheet A from Arg P14 and presumably beyond Ala P9. Moreover, to the best of our knowledge, this is the first instance where the neopeptide of a complexation-specific **monoclonal antibody** has been localized to the loop-inserted part of beta-sheet A, the part of the serpin where the complexation-induced conformational change is most conspicuous.

L10 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 3  
ACCESSION NUMBER: 94164172 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 7509746  
TITLE: Complex formation between **protein C inhibitor** and prostate-specific antigen in vitro and in human semen.  
AUTHOR: Christensson A; Lilja H  
CORPORATE SOURCE: Department of Clinical Chemistry, Lund University, Malmo General Hospital, Sweden.  
SOURCE: European journal of biochemistry / FEBS, (1994 Feb 15) 220 (1) 45-53.  
Journal code: 0107600. ISSN: 0014-2956.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199404  
ENTRY DATE: Entered STN: 19940412  
Last Updated on STN: 20000303  
Entered Medline: 19940401

AB **Protein C inhibitor (PCI), a serine -proteinase inhibitor** first purified from human blood plasma,

occurs at high concentrations (3-4 microM) in seminal fluid in both a high-molecular-mass and low-molecular-mass form. Immunochemical data have previously suggested that PCI in seminal plasma forms complexes with the most abundant **serine proteinase** in semen, prostate-specific antigen (PSA). To provide a structural characterization of the PCI target, immunodetected as PSA, a procedure was developed to isolate low-molecular-mass and high-molecular-mass-forms of PCI from seminal fluid. The high-molecular-mass form of PCI, recognized by **monoclonal antibodies** against PSA, was dissociated by alkaline treatment into the low-molecular-mass form of PCI and a 33-kDa protein identified as PSA by 25 conclusive steps of N-terminal sequence analysis. We developed a sensitive immunofluorometric assay (IFMA) to measure PCI-PSA complexes in body fluids and investigated the rate at which purified PSA may form complexes with purified PCI. Formation of complexes detected by this IFMA and the appearance of SDS-stable approximately 90-kDa complexes paralleled loss of PSA activity recorded with chromogenic substrates. The rate of complex formation was slow compared to that reported for PCI and activated protein C, but was enhanced up to sixfold in the presence of heparin. Less than 10% of the initial PSA activity remained after 3 h incubation with a sevenfold molar excess of PCI and in the presence of heparin. In freshly collected ejaculates, the rate of PCI-PSA complex formation measured by IFMA was similar to that observed between the purified proteins, and paralleled the appearance of SDS-stable complexes by immunoblotting. During gel dissolution in freshly collected ejaculates, approximately 40% of immunodetected PCI becomes complexed to PSA. Although PCI is a slow inhibitor of PSA, complexes between PCI and PSA are detected at levels that correspond to an inactivation of up to 5% of the PSA activity in the ejaculate.

L10 ANSWER 6 OF 7 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 ACCESSION NUMBER: 1993:229242 BIOSIS  
 DOCUMENT NUMBER: PREV199395120417  
 TITLE: Proteolytic inactivation of alpha-1-antitrypsin and alpha-1-antichymotrypsin by neutrophils in arthritic joints.  
 AUTHOR(S): Abbink, Jannie J.; Kamp, Angela M.; Nuijens, Jan H.; Swaak, Tom J. G.; Hack, C. Erik [Reprint author]  
 CORPORATE SOURCE: c/o Publ. Secretariat, Central Lab. Neth. Red Cross Blood Transfusion Serv., P.O. Box 9406, 1006 AK Amsterdam, Netherlands Antilles  
 SOURCE: Arthritis and Rheumatism, (1993) Vol. 36, No. 2, pp. 168-180.  
 CODEN: ARHEAW. ISSN: 0004-3591.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 7 May 1993  
 Last Updated on STN: 8 May 1993  
 AB Objective: In vitro, activated neutrophils create a microenvironment in which proteinase inhibitors are inactivated through the coordinate action of reactive oxygen species and released elastase. We investigated whether such a mechanism may contribute to the destruction of the joint tissues in arthritis. Methods: We analyzed the state of alpha-1-antitrypsin (alpha-1AT) and alpha-1-antichymotrypsin (alpha-1ACT), the two major inhibitors of the neutrophilic **serine proteinases**, in synovial fluid (SF) from patients with inflammatory arthropathies (n = 71) and osteoarthritis (OA) (n = 11), and related the results of neutrophil activation in SF. Results: The ratio of functional to antigenic levels of alpha-1AT in SF patients with inflammatory joint diseases was similar to that of alpha-1AT in normal plasma, whereas that of alpha-1ACT was

significantly decreased. Patients with inflammatory arthropathies had significantly higher levels of inactivated alpha-1AT (i-alpha-1AT) and inactivated alpha-1ACT (i-alpha-1ACT) in SF (as determined with **monoclonal antibodies** specific for the inactivated (i.e., proteolytically inactivated and/or complexed) forms of these inhibitors) than patients with OA (P lt 0.005). Inactivated alpha-1AT and i-alpha-1ACT levels corresponded to 0.3-11% and 3-99%, respectively, of the total amount of these inhibitors in SF. Most of the i-alpha-1AT in SF had a lower M-r than that of native alpha-1AT. Inactivated alpha-1ACT in SF had an M-r identical to that of nonfunctional alpha-1ACT in plasma treated with chymotrypsin. Levels of both i-alpha-1AT and i-alpha-1ACT correlated significantly with lactoferrin and elastase levels. Conclusion: These results suggest that alpha-1AT and alpha-1ACT in arthritic joints are inactivated in part by activated neutrophils, suggesting a role for these cells in impairment of the local balance between proteinases and their inhibitors in arthritis.

L10 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 4  
 ACCESSION NUMBER: 87109153 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 3027058  
 TITLE: Characterization of a cDNA for human **protein C inhibitor**. A new member of the plasma serine protease inhibitor superfamily.  
 AUTHOR: Suzuki K; Deyashiki Y; Nishioka J; Kurachi K; Akira M; Yamamoto S; Hashimoto S  
 CONTRACT NUMBER: HL31511 (NHLBI)  
 SOURCE: Journal of biological chemistry, (1987 Jan 15) 262 (2) 611-6.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-J02639  
 ENTRY MONTH: 198702  
 ENTRY DATE: Entered STN: 19900303  
 Last Updated on STN: 19970203  
 Entered Medline: 19870225

AB A cDNA library in lambda-phage lambda gt11 containing DNA inserts prepared from human liver mRNA was screened with **monoclonal antibodies** to human **protein C inhibitor**. Six positive clones were isolated from 6 X 10<sup>6</sup> phages and plaque purified. The cDNA in the phage containing the largest insert, which hybridized to a DNA probe prepared on the basis of the amino-terminal amino acid sequence of the mature inhibitor, was sequenced. This cDNA insert contained 2106 base pairs coding for a 5'-noncoding region, a 19-amino acid signal peptide, a 387-amino acid mature protein, a stop codon, and a long 3'-noncoding region of 839 base pairs. Based on the amino acid sequence of the carboxyl-terminal peptide released by cleavage of **protein C inhibitor** by activated protein C as well as by thrombin, the reactive site peptide bond of **protein C inhibitor** is Arg354-Ser355. Five potential carbohydrate-binding sites were found in the mature protein. The high homology of the amino acid sequence of **protein C inhibitor** to the other known inhibitors clearly demonstrates that **protein C inhibitor** is a member of the superfamily of serine protease inhibitors including alpha 1-antichymotrypsin, alpha 1-antitrypsin, antithrombin III, ovalbumin, and angiotensinogen. Based on the difference matrices for these proteins, we present possible phylogenetic trees for these proteins.